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Primer Note

Isolation of 16 microsatellite markers for *Spiraea alpina* and *S. mongolica* (Rosaceae) of the Qinghai–Tibet Plateau[1]

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- *Premise of the study:* A set of microsatellite markers were developed to characterize the level of genetic diversity and gene flow in two plant species endemic to the Qinghai–Tibet Plateau, *Spiraea alpina* and *S. mongolica*.
- *Methods and Results:* Using the Fast Isolation by AFLP of Sequences COntaining repeats (FIASCO) method, 16 microsatellite loci showed polymorphisms in both species. In two populations of each species, the number of alleles per locus ranged from three to 18 in *S. alpina* and from four to 30 in *S. mongolica*.
- *Conclusions:* These microsatellite markers provide an efficient tool for population genetic studies and will be used to assess the genetic diversity and spatial genetic structure of *S. alpina* and *S. mongolica.*

Key words: gene flow; genetic diversity; microsatellite markers; population genetics; Qinghai–Tibet Plateau; *Spiraea*.

Spiraea alpina Pall. and *S. mongolica* Maxim. (Rosaceae subfam. Spiraeoideae) are perennial shrubs, found in western China and some areas of Mongolia and Siberia. The two alpine plants usually grow on sunny slopes or ridges. They are widespread across the Qinghai–Tibet Plateau and adjacent highlands, at altitudes of 2000–4500 m (Lu et al., 2003). Due to high levels of morphological variation, the genus *Spiraea* L. has been classified in several ways by different authors into various subgenera, sections, and series (Lu et al., 2003; Potter et al., 2007). A recent phylogeographic analysis of cpDNA variations in *S. alpina* indicated that this alpine shrub survived in multiple refugia during the Last Glacial Maximum and that earlier glaciations may have triggered deep intraspecific divergence (Zhang et al., 2012). However, the phylogeographic analysis based on one uniparentally inherited cpDNA fragment may only partly recover the phylogeographic history of a species. Biparentally inherited simple sequence repeat (SSR) markers with more polymorphism and information are necessary for a better understanding of the genetic structure and phylogeographic history of *S. alpina* and *S. mongolica.* In this study, we isolated 16 polymorphic microsatellite primers to facilitate the investigation in further studies for these two species.

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METHODS AND RESULTS

Total genomic DNA was extracted from silica gel–dried leaves of *S. alpina* following the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Microsatellite loci from an enriched $(AG)_n$ library were isolated using the Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) method with minor modifications (Zane et al., 2002). Approximately 300 ng of genomic DNA were completely digested with *Mse*I (New England Biolabs, Beverly, Massachusetts, USA), and then ligated to an *Mse*I AFLP adapter (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′) using T4 DNA ligase (New England Biolabs). The diluted digestion-ligation mixture (1 : 10) was amplified with adapter-specific primers (5′-GATGAGTCCTGAGTAAN-3′). For enrichment, the PCR products were denatured at 95°C for 5 min, then hybridized with two 5′-biotinylated probes, $(AC)_{15}$ and $(AG)_{15}$, respectively, in a 250-μL hybridization solution (4× saline sodium citrate [SSC], 0.1% sodium dodecyl sulfate [SDS], 0.5 μmol/L probe) at 48°C for 2 h. Streptavidin-coated magnetic beads (New England Biolabs) were used to separate and capture the DNA fragments hybridized to the probe at room temperature for 20 min, followed by two washing steps: three times in TEN_{100} (10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl [pH 7.5]) for 8 min and three times in TEN_{1000} (10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl [pH 7.5]) for 8 min. The separated single-stranded DNA fragments were amplified with adapter-specific primers as described above. The PCR products, after purification using a CASpure PCR Purification Kit (Sangon, Shanghai, China), were ligated into the pGEM-T Easy Vector (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's instructions, then transformed into *Escherichia coli* TOP10 competent cells (Trans Gen Biotech, Beijing, China). Transformants were plated, and insert-containing clones were selected by blue-white screening with ampicillin, X-Gal, and isopropyl-β-D-1-thiogalactopyranoside (IPTG). Positive clones were tested by PCR using $(AC)_{10}$/$(AG)_{10}$ and $M13^+$/ $M13^-$ as primers.

